Agilent Genomic Workbench Feature Extraction 10.9

Quick Start Guide

What is Agilent Feature Extraction software? 2
Getting Help 4
What's new in version 10.9? 7
Starting the program and setting up eArray 9
Setting up a Standard Project to extract Agilent and non-Agilent image files 16
Setting up an On-Time project to extract Agilent image files in real time 42

This Quick Start Guide provides an overview of how to use the Feature Extraction 10.9 software, with step-by-step instructions on how to start the program and run an extraction. Exercises are also provided to help you, through the use of example images provided with the software.

This guide assumes you have already installed Feature Extraction 10.9 and its license on your computer. Feature Extraction 10.9 is installed automatically as part of the Agilent Genomic Workbench Standard Edition. If you need to install the software, see the installation guide for the Agilent Genomic Workbench Standard Edition.

For a complete description of the Feature Extraction main window and its components, see the *Feature Extraction 10.9 Users Guide*.

Agilent Technologies

What is Agilent Feature Extraction software?

Agilent Feature Extraction 10.9 software automatically reads and processes up to 100 raw microarray image files in the batch mode, or real-time extraction as soon as the .tif image is produced by the Agilent scanner. It finds and adjusts microarray grids, rejects outlier pixels, accurately calculates feature intensities and ratios, flags outlier features, and calculates statistical confidences. Application-specific QC reports summarize multiple analyses.

Feature Extraction is a key component of Agilent Genomics Workbench, a comprehensive informatics platform that integrates complementary technologies to provide a complete genomic solution, from design of microarray and target enrichment libraries through analysis and reporting.

Agilent Feature Extraction software extracts data from microarray images produced in two different situations:

- Agilent microarrays scanned on an Agilent scanner
- Non-Agilent microarrays scanned on an Agilent scanner

Feature Extraction handles each of these images differently. These differences are described throughout the documentation.

NOTE Feature Extraction versions 10.7 and higher do not support microarray images generated by the GenePix/Axon scanner.

If you are a new user

Read "Getting Help" on page 4.

"Starting the program and setting up eArray" on page 9.

If you intend to analyze...

- Saved Agilent images, read the "Instructional overview" on page 16, and go to "Example Exercise 1: Set up and run batch extractions on Agilent images" on page 26.
- Saved non-Agilent images, read the "Instructional overview" on page 16, and go to "Example Exercise 2: Set up and run batch extractions on non-Agilent images" on page 38.

Agilent images in real time, read the "Instructional overview" on page 42, and go to "General Instructions" on page 43.

If you are an experienced user

Read "What's new in version 10.9?" on page 7.

Do the exercises again if you want to sharpen your skills.

To manually download updates

When Feature Extraction is installed, Feature Extraction protocols and QC metric sets are also installed. Grid templates are not included with the installation. But if you set up an eArray login in Feature Extraction, the software will automatically download and update the necessary grid templates and Feature Extraction protocols from the eArray server. You can also go to the following web sites to download updates manually.

Listed below are some useful Agilent Web sites:

www.agilent.com/chem/earray - To register for eArray account

www.agilent.com/chem/feprotocols - To download Feature Extraction protocols

www.agilent.com/chem/designfile - To download grid templates

www.agilent.com/chem/feqcmetrics - To download QC metrics

www.agilent.com/chem/registration - To subscribe to E-Notes for monthly updates on Agilent products

Getting Help

To get help for Feature Extraction

The following tables list the resources available for getting help. Guides and manuals are opened using Adobe® Reader® software.

Help Resource	Description/Instructions
Feature Extraction Quick Start Guide	This guide, which you are reading, gives you an overview of the Feature Extraction software, including tutorial exercises. To open this guide from within Feature Extraction, click **Help > Quick Start Guide**.
Feature Extraction User Guide	A comprehensive guide for using the Feature Extraction software, that includes the following chapters: • Chapter 1– Working with Feature Extraction • Chapter 2– Extracting Microarrays Automatically • Chapter 3– Creating Grid Files and Templates • Chapter 4– Changing Protocol Settings • Chapter 5– Changing Image Displays To open this guide from within Feature Extraction, click **Help > User Guide**.
Feature Extraction Reference Guide	A reference guide that contains information about protocols, QC reports, results, and algorithms, that includes the following chapters: • Chapter 1 – Default Protocol Settings • Chapter 2 – QC Report Results • Chapter 3 – Text File Parameters and Results • Chapter 4 – MAGE-ML Results • Chapter 5 – How Algorithms Calculate Results • Chapter 6 – Command Line Feature Extraction To open this guide from within Feature Extraction, click **Help > Reference Guide**.
Technical Support	Opens a link to the Agilent Technical Support Web site where you can find literature and manuals, view Frequently Asked Questions, and download updates and revision information. There is also a **Contact Us** link where you can locate the technical support phone number or send an e-mail to technical support for your country.

Help Resource	Description/Instructions
Feature Extraction Training	Opens a link to the Feature Extraction Tutorials Web Site, where you can view a number of self-paced instructional tutorials. To open this Web site from within Feature Extraction, click **Help > Feature Extraction Training**.
Feature Extraction on the Web	Opens a menu that provides links to the following: • **Array annotation** – Find available design files by barcode • **Feature Extraction Software** – Feature Extraction software downloads and information • **Feature Extraction Protocols** – Download current and previous versions of Feature Extraction protocols
Agilent License	Opens the Agilent software license redemption Web site, where you use your entitlement certificate to redeem your software license.
Memory Status	Opens an information window that shows how much computer memory is currently available and how much memory is in use.
About	Opens an information window that shows your Feature Extraction version, license, and database information. This window also displays your computer Host ID, which you need in order to redeem your license.

To get help within Agilent Genomic Workbench

Help Resource	Description/Instructions
Feature Extraction Guides	From the Help tab in Agilent Genomic Workbench, a Feature Extraction button appears if the selected application can use Feature Extraction. Click **Feature Extraction** to open a menu that lets you open the following guides: • Feature Extraction Quick Start Guide • Feature Extraction User Guide • Feature Extraction Reference Guide

Help Resource	Description/Instructions
Other User Guides	The Help tab in Agilent Genomic Workbench lets you view any of the available user guides that apply to the currently selected application type. **1** Set the desired application type from the Switch Application menu. **2** In the Agilent Genomic Workbench tab bar, click **Help.** The names of the available user guides appear in the command ribbon. **3** Click the desired user guide. The selected user guide opens in Adobe Reader.

To learn about Agilent products and services

To view information about the Life Sciences and Chemical Analysis products and services that are available from Agilent, go to www.chem.agilent.com.

What's new in version 10.9?

Feature Extraction 10.9 includes the following new and changed functions:

- Installation of Feature Extraction is now done through Agilent Genomic Workbench.
- MySQL database is fully integrated with Agilent Genomic Workbench.
- QC tools are now fully integrated with Agilent Genomic Workbench.
- New STATS have been added to the tab text output file.
- At the end of an extraction, no Run Chart dialog box is displayed.
- Feature Extraction backup and restore is no longer available.

See the Release Notes installed with the Feature Extraction software for more information about Feature Extraction 10.9 (**Start > Programs > Agilent > Agilent Genomic Workbench <version>\FeatureExtraction > Release Notes**).

Installation now done through Agilent Genomic Workbench

Feature Extraction software is now installed using the Agilent Genomic Workbench installer. Once installed, Feature Extraction can be started with the Feature Extraction desktop icon, from the Windows Start menu, or it can be started from within the Agilent Genomic Workbench application. The license can also be entered from Agilent Genomic Workbench.

MySQL database fully integrated with Agilent Genomic Workbench

Starting with version 10.9, Feature Extraction shares the MySQL database with Agilent Genomic Workbench. This lets the programs share microarray and probe data, grid templates/designs, Feature Extraction parameter protocols, and QC metric sets in one centralized database.

QC tools are now integrated with Agilent Genomic Workbench

QC tools for creating metrics and charts are no longer a separate application, and are part of the Quality module in Agilent Genomic Workbench. See the Agilent Genomic Workbench *Quality Tools User Guide* for more information.

New STATS are now part of the tab text output file.

The following STATS are part of the tab text output file:

- Report AF Hold
- PMT Volts
- Glass thickness
- Image depth (16bit or 20bit)

These STATS are also available for creating metrics in the Quality tab of Agilent Genomic Workbench.

Feature Extraction backup and restore no longer available

Because Feature Extraction now shares the database with Agilent Genomic Workbench, the Feature Extraction backup and restore function is no longer available.

To move custom protocols and QC metric sets from Feature Extraction 10.7 to Feature Extraction 10.9, you must export them in Feature Extraction 10.7 and then import them to Feature Extraction 10.9. See the *Feature Extraction Software 10.9 User Guide* and the Agilent Genomic Workbench *Quality Tools User Guide* for more information. See the Agilent Genomic Workbench Standard Edition *Installation Guide* for information on how to back up and restore the Agilent Genomic Workbench database.

Starting the program and setting up eArray

The Feature Extraction program can be started in one of two ways:

- From Microsoft® Windows®
- From the Agilent Genomic Workbench Open Application tab

To start the program from Windows

- Double-click the **Feature Extraction** shortcut on your desktop.

  This shortcut was created when the Feature Extraction program was installed.

  OR

- From Windows, click **Start > All Programs > Agilent Genomic Workbench Standard Edition<revision> AGW Feature Extraction <revision>**.

To start the program from Agilent Genomic Workbench

1. Open Agilent Genomic Workbench, if you have not already done so.
2. In the Agilent Genomic Workbench main window, click the **Open Application** tab.
3. In the Open Application tab, click the Feature Extraction icon

.

NOTE You must have a valid license installed in order to use Feature Extraction. If you do not have a license installed, you will see an error message.

To set up a Feature Extraction license

Redeem your software licenses

To obtain your Feature Extraction license:

1 Start the Feature Extraction program and select **Help > Agilent License**.

Have the following items available:

- **Software Entitlement Certificate (SEC)** - This PDF contains the Order Number and Certificate Number that you need to provide in order to redeem your purchased license.
- **Host ID** - This can be found under the menu Help > About. You need to provide the host ID to redeem your purchased license.

2 Follow the instructions on the Agilent Software License Redemption Web site to redeem your purchased license.

After redeeming the license online, you will receive an e-mail with the license attached.

3 Save the unzipped license file to the folder on the drive where you installed the software. By default, Feature Extraction is installed in the Agilent Genomic Workbench installation folder:

C:\Program Files\Agilent\Agilent Genomic Workbench Standard Edition <version>\FeatureExtraction

Set up the license from Agilent Genomic Workbench

1 Locate your license file and double-click it to open the file in Notepad.

2 Highlight the license text, and copy it to the Clipboard.

3 Start the Agilent Genomic Workbench software. See "To start the program from Agilent Genomic Workbench" on page 9.

4 In the Open Application tab in Agilent Genomic Workbench, next to Feature Extraction, click **License**.

5 Click **License** and then paste the license in the blank license field. (To paste the text, press **Ctrl V** on your keyboard.)

Set up the license from Feature Extraction

1 Start the Feature Extraction software, if it is not already running.

If the Feature Extraction program is already running (unlicensed), click the **New Project** icon .

2 A message appears that informs you that you do not have a valid license installed. Click **Retry**.

The Select Agilent Feature Extraction License File dialog box opens.

3 Find the directory that contains the license file.

4 Select the license file (.lic), and click **Open**.

To set up eArray login

When you first open Feature Extraction, the **eArray Login Setting** window appears on top of the Feature Extraction main window. If you do not see the eArray Login Setting window, you can access it using Tools > eArray settings.

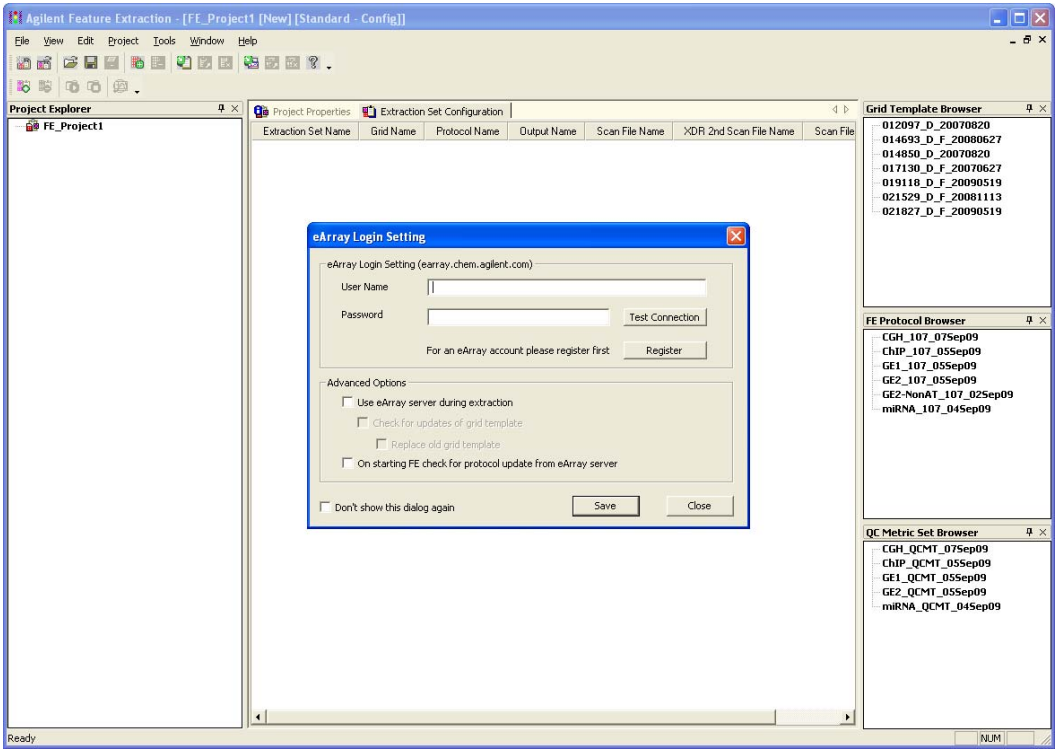


Figure 1 eArray Login Setting dialog box

Feature Extraction connects to the Agilent eArray server to automatically download, install, and use the required grid templates, Feature Extraction protocols, and QC metric sets during extraction.

For information on the eArray server and how to use the $\text{eArray}_{\text{XD}}$ module in Agilent Genomic Workbench to create and manage custom microarray content for CGH, ChIP, methylation, gene expression, and microRNA applications, see the *$eArray_{XD}$ User Guide*.

It is highly recommended that you set up an eArray login before you run the extraction project to take advantage of this feature.

NOTE An internet connection is required in order to log into eArray.

1 Type your eArray **User Name** and **Password** in the eArray Login Setting window, if you already have an existing eArray account. Hint: The user name is the e-mail address you provided when you created an eArray account.

   If you do not have an eArray account, you must first register for one. To start the registration process, click **Register**. This will take you to the Agilent eArray registration Web site where you can request a free eArray account. Follow the instructions provided on the eArray Web site to complete the registration.

2 Click **Test Connection**. The message "Login Successful!!!" is displayed if the connection to the eArray server is successful. If you get the message "Login denied", check that the user name and password you typed are correct.

3 Under Advanced Options, select the appropriate boxes:

- **Use eArray server during extraction**

  Select this check box to log into eArray server to automatically download, install, and use grid templates (design files), Feature Extraction protocols, and QC metric sets during extraction.

- **Check for updates of grid template**

  Select this check box to download updates to existing grid templates already in the Grid Template Browser.

- **Replace old grid template**

  When you select this check box, Feature Extraction silently replace old grid templates in the Grid Template Browser with the latest ones from the eArray server.

- **On starting Feature Extraction check for protocol update from eArray server**

  Select this box to download updates from the eArray server for existing default protocols and QC metric sets in the FE Protocol Browser.

4 To save the eArray login settings, click **Save**. You will get a message "Setting updated successfully". Click **OK** to close the message, and click **Close** to exit the eArray Login Setting window.

   Now you see the Feature Extraction main window.

To open an image file

1 With the Feature Extraction main window open, click **File > Open > Image**, and browse to the folder that contains the image file. Double-click the image file (.tif) that you want to open.

If the Feature Extraction program is running, you can also "drag and drop" the image from your folder to the open Feature Application window.

If you do not have the Feature Extraction program running, you can also open an image file by dragging and dropping an image file to the Feature Extraction shortcut on your desktop.

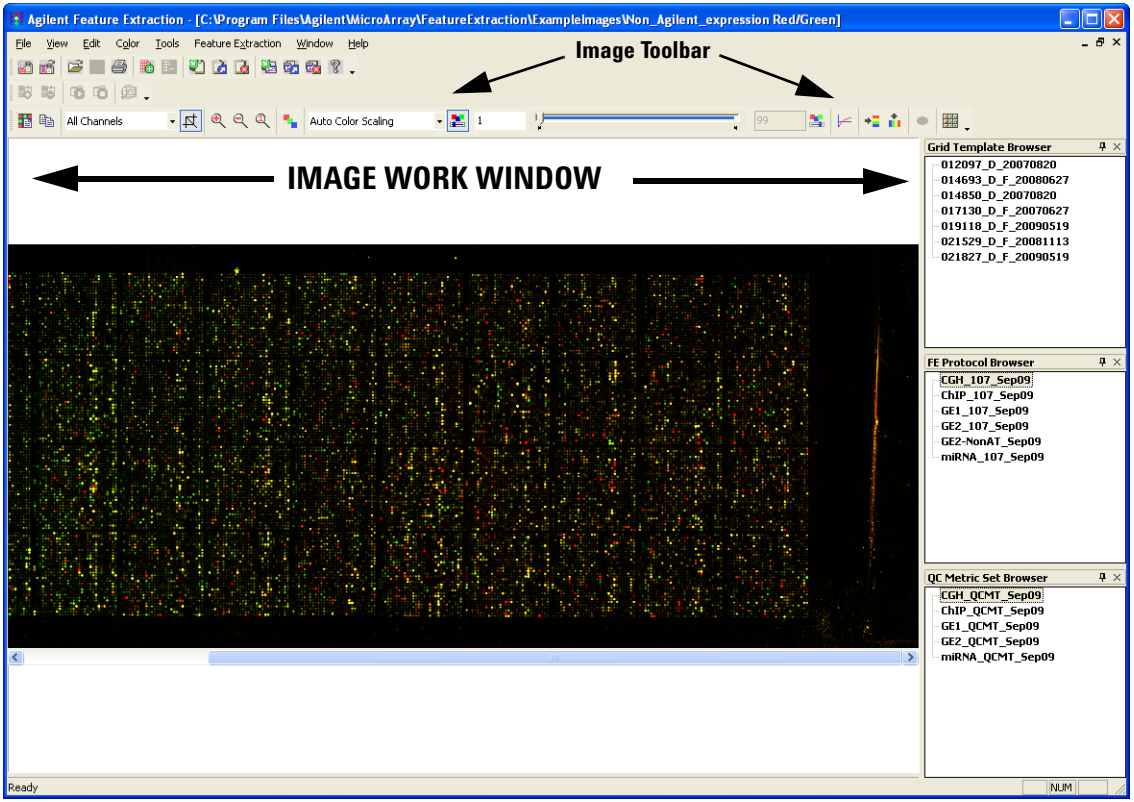


Figure 2 Feature Extraction main window with open image

NOTE

For high resolution images (2 or 3 micron or 20-bit), you see a low-resolution preview image until you crop to roughly 1/10 of the preview image size. Then you see the original high-resolution image. [The Crop Cursor turns red when you are outside the high-resolution (High Fidelity) region.]

To learn more about interactively finding spots and creating grid files for non-Agilent images, see Chapter 3, “Creating Grid Files”, in the *Feature Extraction 10.9 User Guide*.

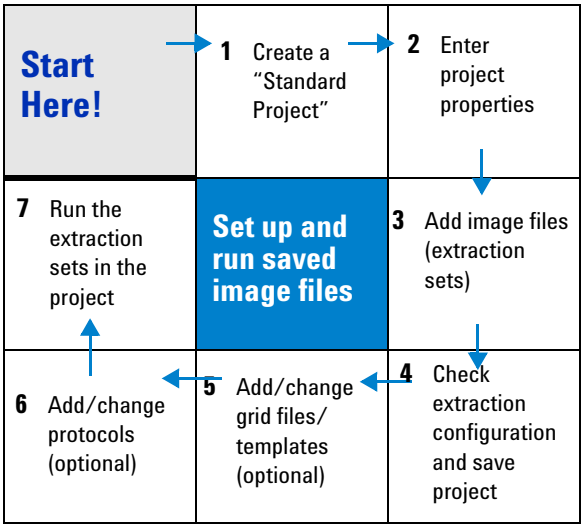
To learn more about changing the image display, see Chapter 5, “Changing Image Displays”, in the *Feature Extraction 10.9 User Guide*.

Setting up a Standard Project to extract Agilent and non-Agilent image files

Instructional overview

Follow the road map below and the instructions on the next pages to get started.

See Chapter 2 in the *Feature Extraction 10.9 User Guide* for details on this task.


Start Here!
1 Create a "Standard Project"
2 Enter project properties
3 Add image files (extraction sets)
4 Check extraction configuration and save project
5 Add/change grid files/ templates (optional)
6 Add/change protocols (optional)
7 Run the extraction sets in the project
Set up and run saved image files

The Agilent Feature Extraction program works with Agilent or non-Agilent microarray images produced on Agilent scanners using projects, extraction sets, grid templates or files, and protocols. The images are organized into extraction sets that contain the grid and protocol information to run the extraction. The extraction sets, in turn, are organized into projects.

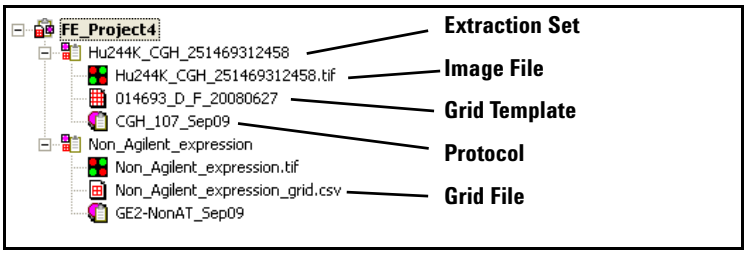


Figure 3 Project Explorer with expanded extraction sets

project A grouping of information that contains one or more *extraction sets* that define the files to use in the extraction.

extraction set Each group that contains a microarray image file, grid template (or grid file) and protocol within a project. This term is used both before and after extraction.

grid template Grid information from Agilent design files or grid files stored in the database. A grid template includes feature annotation and general geometry about the microarray (number of rows, columns, subgrids, feature spacings), which is used to find spots before data analysis takes place. Although not specific to any microarray image, grid template information is usually applied to the image for which the template was designed.

grid file Grid files are required in order to do feature extraction for non-Agilent images. Rarely, the automatic grid placement of Agilent images using a grid template may produce an incorrect grid, which will also require the use of a user-generated grid file. When using a grid file, extraction uses the nominal and "found" spot locations specific to the image from which the grid file was created. Grid files are created in the Feature Extraction program using a microarray image file and a microarray layout file (such as an Agilent Design file or a .GAL [GenePix Array List] file). The grid file contains the locations of the "found" spots if the "Calculate spot size and centroids" option is run before the file is saved. You can create grid templates from grid files, although the templates have only geometry information, no found spots.

protocol A list of steps and parameter values that define the data analysis algorithms and calculations used for feature extraction of a microarray image or images.

General Instructions

Step 1. Create a Standard Project

- If the Feature Extraction program is not already running, double-click the **Feature Extraction** shortcut on your desktop to start, or click the **Feature Extraction** icon in the Open Application tab of Agilent Genomic Workbench.

  Feature Extraction automatically creates a standard project (FE_Project1) every time you start the program. The Feature Extraction main window appears with the Project1 work window and an empty Extraction Set Configuration tab window.

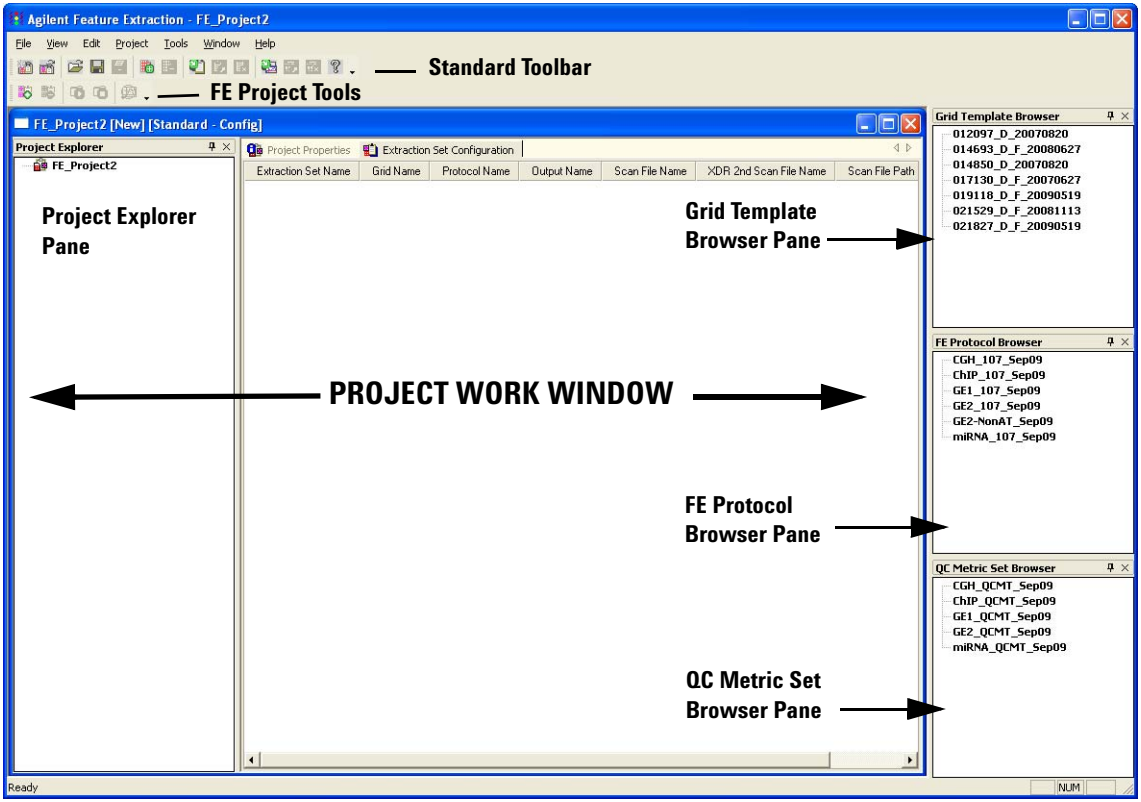


Figure 4 Feature Extraction main window

You can change where and how the project work window and the browser panes dock when you start the program. To learn more about working with this interface, see the *Feature Extraction 10.9 User Guide*.

Step 2. Enter the project properties

1 Click the **Project Properties** tab to view the default project properties.

2 To change a default setting, select a property and click the down arrow that appears on the right column. Choose from the available options.

⊟ General	
Operator	Unknown
⊟ Input	
Number of Extraction Sets Included	0
⊟ Output and Data Transfer	
⊟ Outputs	
⊞ MAGE	None
JPEG	None
⊟ TEXT	Local file only
Output Package	Compact
Visual Results	Local file only
Grid	None
QC Report	Local PDF file only
FTP Send Tiff File	False
⊟ Local File Folder	
Same As Image	True
Results Folder	
⊞ FTP Setting	
⊟ Automatic Protocol Assignment	
Highest Priority Default Protocol	Grid Template Default
Project Default Protocol	
⊟ Automatic Grid Template Assignment	
Use Grid file if available	False
External DyeNorm List File	
Overwrite Previous Results	False

Figure 5 Default project properties for a standard project

You can run Feature Extraction for one or more Agilent images generated by the Agilent scanner with these default project properties. You can also set up and run multiple extractions with a combination of Agilent and non-Agilent images.

To learn more about setting up and running batch extractions with the Project Work Window, see the exercises in this guide:

- "Example Exercise 1: Set up and run batch extractions on Agilent images" on page 26.
- "Example Exercise 2: Set up and run batch extractions on non-Agilent images" on page 38.

Step 3. Add image files (extraction sets) to the project

1 In the Project Explorer, right-click **FE_Project1**, and click **Add Extraction**.

2 Select one or more images, and click **Open**.

- To select multiple images at a time, hold down the **Ctrl** key and select the images.
- You can also add one or more image files by dragging and dropping them from the folder to the open project in the Feature Extraction window.

Any extraction set that is missing the required components, such as grid template or protocol, shows a lightning bolt in Project Explorer and a **"?"** in the icon of the missing component. (Provided eArray is enabled and the image is an Agilent image.) In versions prior to 10.5, if any of the required components were not attached to the tiff image, then the extraction set would not be extracted. This changed beginning with version 10.5.

You do not need to assign a grid template/grid file and a protocol to an image file in order to run the extraction. Extraction sets without a grid template and/or a protocol attached to the project can be extracted, by setting the program to automatically determine the correct template and protocol to use. For this functionality to work, **one** of the following must be true:

- Prior to running the extraction, you set up to use the eArray server during extraction, as discussed in the section "To set up eArray login" on page 11.
- Prior to running the extraction, the required grid templates and protocols already exists in the database and are shown in the Grid Template Browser and FE Protocol Browser.

For custom grid templates and protocols that cannot be found on the eArray server, you must add them manually to the database before you run the extraction. See the *Feature Extraction 10.9 User Guide*.

If grid templates are not loaded automatically, or you want to use a different grid template or protocol, you can assign them manually. See Step 4 "Assign grid template/grid file to the extraction set (depending on type of images)" on page 21 and Step 5 "Assign a protocol to the extraction set (optional)" on page 22.

Step 3. Assign grid template/grid file to the extraction set (depending on type of images)

Agilent images By default, if the required grid templates are in the database, when you add Agilent tiff images to the project, Feature Extraction will automatically associate the appropriate grid templates to the extraction sets. You can also assign a grid template or grid file to the Agilent image.

To assign a grid template for Agilent images:

1. Expand the extraction set.
2. Select a grid template from the Grid Template Browser, hold down the mouse button and drag it to the second position of the extraction set in Project Explorer.
3. Let go of the mouse button to drop it at this position.

To assign a grid file for Agilent images:

1. Right-click the second position of the expanded extraction set and click **Select Grid File**.
2. Select the grid file, and click **Open**.

Non-Agilent images You must assign a grid file to non-Agilent images in the project. The extraction automation feature is not supported for non-Agilent images. Feature Extraction will not extract non-Agilent images that are missing grid files or protocols.

To assign a grid file for non-Agilent images:

1. Right-click the second position of the expanded extraction set and click **Select Grid File**.
2. Select the grid file, and click **Open**.

AMADID is the Agilent MicroArray Design IDentifier.

Agilent microarray images scanned with an Agilent scanner For Agilent microarray images with barcode information included in the image, the program selects a grid template from the database using the AMADID portion of the barcode associated with the image file. If there are grid templates for that AMADID that come from multiple design files, the program selects the grid template from the most recently created Agilent design file. To add a barcode if the image has none, see the *Feature Extraction 10.9 User Guide*.

For this automated assignment to work, the grid template must exist in the database. If it does not, you can let the Feature Extraction try to automatically download the required grid template from the eArray server or you can interactively associate a grid template or grid file with the extraction set. See "Agilent images" on page 21 for details on how to assign a grid template or grid file.

Agilent uses barcode information for many workflow automation and data tracking steps. Inclusion of the Agilent barcode information in the TIFF file helps ensure that the data is quickly, easily and accurately processed and that informatics programs know how to analyze the results.

Non-Agilent images scanned with an Agilent scanner Non-Agilent image files from an Agilent scanner do not point to grid information that the Agilent Feature Extraction program can read. Therefore, when you load the non-Agilent image file, you must add a grid file that you have already created.

See Chapter 3 in the *Feature Extraction 10.9 User Guide* to learn how to create grid files.

Step 3. Assign a protocol to the extraction set (optional)

Automatically assigned protocols come from two sources: a default protocol associated with the grid template, or a project level default protocol. You select which of these options has the higher priority. (See Chapter 2 of the *Feature Extraction 10.9 User Guide* to learn how to associate a protocol with a grid template and where to enter the project level default protocol.) You can also add a protocol to the extraction set manually.

To add a protocol manually:

1. From the Protocol Browser select a protocol, hold down the mouse button and drag it to the third position of the extraction set.
2. Release the mouse button to drop the protocol onto the third position of the set.

Feature Extraction can run analyses on Agilent 1-color gene expression (GE1), 2-color gene expression (GE2), CGH, ChIP, miRNA, and non-Agilent 2-color GE (GE2-nonAT) microarray images scanned on the Agilent scanner. Agilent includes Feature Extraction protocols for extracting each of these image types.

CAUTION These Feature Extraction Protocols were optimized using data from Agilent catalog arrays, which have many replicated probes and validated Negative Control probes. If custom arrays without enough replicated probes are used, or arrays with custom probes designated as Negative Control probes, the default Feature Extraction Protocols may not be optimal. The Feature Extraction Protocol optimization also uses Agilent's default lab protocols; that is, label, hybridization, wash, and scanner procedures.

Step 3. Add or change grid templates from design files (optional)

For the Agilent images you intend to extract, you add grid templates based on the images' design files to the Grid Template Browser.

1 Right-click the Grid Template Browser pane, and click **Add**.

2 Select a design file or files, and click **Open**.

Every extraction set must have an image file, assigned grid template/grid file and protocol. Grid templates and protocols for Agilent images are loaded to the extraction set automatically by the program, if the grid template associated with the loaded image's design file is available in the database. If a grid template is in the database, it is displayed automatically in the Grid Template Browser. If a grid is not in the database, the program will automatically get the grid template from eArray when the extraction starts. For details on how to update or add grid templates using Agilent eArray, see the *Feature Extraction 10.9 User Guide*.

Step 3. Change QC metric set associated with the protocol to monitor QC metrics

At times the Feature Extraction program is used in a production environment, where the microarray processing protocols are similar and monitoring run-to-run consistency is an important goal. Feature Extraction can help monitor this consistency with the QC metric sets.

In earlier versions of Feature Extraction, you choose one QC metric set in the Project Properties tab that was used for all extractions in a project.

In version 10.5 and higher, the QC metric sets and protocols are linked. The Feature Extraction protocol defines which metric set is used with it.

To change the QC metric set associated to the Feature Extraction protocol:

1 In the FE Protocol Browser, double-click a protocol to open the FE Protocol Editor.

2 Select **Protocol Properties** to see the protocol properties.

3 Under General, click the cell next to **Metricset**.

4 Click the down arrow, and select the QC metric set appropriate for your microarray application.

   If a metric set you want to use is not available in the metric set list, you must first import it to the database.

   To import a QC metric set into the database:

   1 Select **View > QC Metric Set Browser** from Feature Extraction main window, if it is not already visible.

   2 The QC Metric Set Browser displays all of the default metric sets and custom metric sets in the database. Custom metric sets are created using the Quality module in Agilent Genomic Workbench. Right-click in the QC Metric Set Browser and select **Import** to import metric sets that are not shown in the QC Metric Set Browser.

   The default QC metric sets installed with the Feature Extraction software are read-only and cannot be removed from the database. However, you can remove or change a QC metric set associated with the protocol and then save it as a new protocol with a different name.

   To remove a QC metric set from the protocol:

   - Select **<Set empty>** from the metric set list in the Protocol Properties.

5 Click **Save As** to save the new protocol.

QC metric sets Metric sets are loaded into the database when you install the Feature Extraction software. If you set up the eArray login as discussed in the section "To set up eArray login" on page 11, then Feature Extraction will automatically download and install the latest QC metric sets from the eArray server.

If you remove a QC metric set, updates of QC metric sets are also available at www.agilent.com/chem/feqcmetrics, if you need to manually download and install them in Feature Extraction.

CAUTION Agilent-supplied QC metric sets are intended to assist users in monitoring microarray processing issues. They were not developed to detect microarray manufacturing issues.

When you create QC metric sets using the Agilent Genomic Workbench Quality tools, you can associate them to protocols in Feature Extraction. When that project is run, the Feature Extraction program summarizes the metric statistics on each microarray's QC report and shows how the results compare to the thresholds (if any) defined in the metric set. It also reports this information in the output files in the statistics table. For more information on using the Quality module in Agilent Genomic Workbench, see the Agilent Genomic Workbench *Quality Tools User Guide*.

Step 6. Save the project

1 Select **File > Save As**.

By default, your projects are saved in **C:\Program Files\Agilent\Agilent Genomic Workbench Standard Edition <version>\FeatureExtraction\FEProjects**.

2 To save in a different location, go to or create the folder for your projects.

3 Type a project name, and click **Save**.

Step 4. Run Feature Extraction

1 Click the **Project Run mode On/Off** button on the Feature Extraction Project toolbar , or select **Project > Start Extracting**.

Example Exercise 1: Set up and run batch extractions on Agilent images

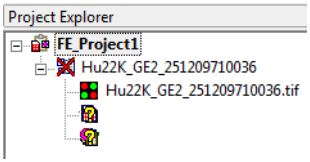
The following exercise takes you through the setup, analysis, and reporting steps of Feature Extraction with example images provided by Agilent. These images are provided on the Agilent Genomic Workbench installation disk.

With this exercise you learn how to:

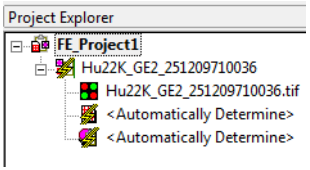
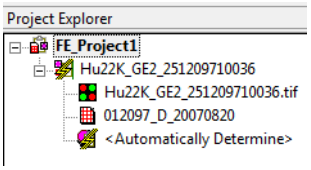
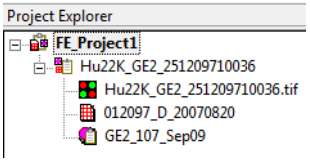
- Set up and extract a 2-color GE microarray image and produce a 2-color GE QC report with spike-ins.
- Set up and extract four 1-color GE images on a 4-multiplex microarray and produce a 1-color GE QC report.

Try the Steps on the left before reading the Detailed Instructions.

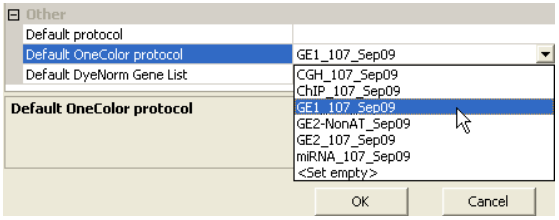
Task 1: Add an extraction set for a 2-color Gene Expression microarray image file

Steps	Detailed Instructions	Comments
1 Add the 2-color gene expression image file that was installed with the Feature Extraction software to Project 1.	**a** Start the Feature Extraction program. **b** Click the **Add Extraction Set(s)** button on the toolbar, or right-click Project Explorer, and select **Add Extraction.** **c** The example images are located in the installation folder for the Agilent Genomic Workbench client. By default, this is **C:\Program Files\Agilent\Agilent Genomic Workbench Standard Edition <version>\FeatureExtraction\ ExampleImages**. Select the **Hu22K_GE2_251209710036.tif** file, and click **Open**. **d** Expand the extraction set that appears in Project Explorer. 	• There may be times when you need to manually assign a grid template and/or a protocol to the extraction set. Task 1 is intended to show you how to do this. • The grid template associated to this image is automatically assigned to the extraction set when the grid template is available in the Grid Template Browser.

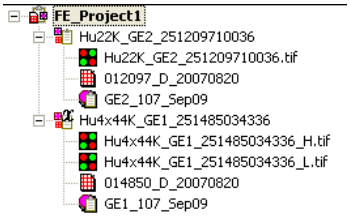
Task 1: Add an extraction set for a 2-color Gene Expression microarray image file (continued)

Steps	Detailed Instructions	Comments
2 Add a grid template to the extraction set, if one has not been automatically assigned. Note: The grid template for this image is a design file that begins with **012097_D...**	**a** Right-click the Grid Template Browser, and click **Add**. **b** Go to the ExampleImages folder, or the folder where the image file is stored. **c** Select the design file (**012097_D_20070820.xml)**, and click **Open**. **d** When the message appears that tells you the grid template was successfully added, click **OK**.  **e** Drag and drop the new grid template to the second row, after the image name. 	• The program will automatically assign the appropriate grid template and protocol for the image if <Automatically Determine> is displayed in the extraction set, and you have selected in the eArray settings to use the eArray server during extraction.
3 Assign the gene expression 2-color protocol to the extraction set. Note: The 2-color GE protocol for this extraction set has spike-ins turned on as the default setting. The QC report will show all the spike-in results.	• From the FE Protocol Browser, drag and drop the **GE2_107_Sep09** Feature Extraction protocol to the third row of the gene expression extraction set. 	• You can add a GE2_105 (version 10.5) Feature Extraction protocol to the Grid Template Browser if you want to compare data run on v10.5 vs. v10.9. The extraction set using v10.5 Feature Extraction protocol is analyzed using v10.5 data processing, but with v10.9 for image processing. • The latest protocols can be automatically downloaded from eArray server and installed on Feature Extraction, if you select this in the eArray settings.

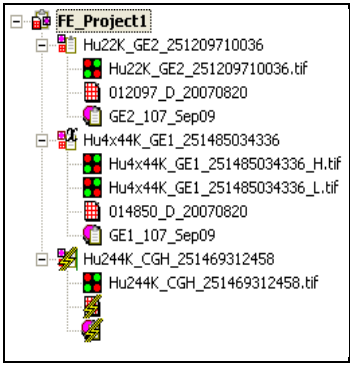
Task 2: Add an extraction set for a 1-color Gene Expression image file of 4-multiplex microarray

Steps	Detailed Instructions	Comments
1 Add a grid template from the design file. Note: The design file for this image is 014850_D_20070207.xml.	**a** Right-click the Grid Template Browser, and click **Add**. **b** Go to the directory where the image file is stored. **c** Select the design file (**014850_D_20070820.xml**), and click **Open**. **d** When the message appears that tells you the design was added successfully, click **OK**.	• Task 2 shows you how to associate a default Feature Extraction protocol with a grid template. When the image and grid template are loaded in the project, Feature Extraction automatically attaches the default protocol to the extraction set.
2 Assign a default 1-color GE protocol to the grid template to use for the microarray image with barcode 25148503446.	**a** Right-click grid template **14850_D_20070820** in the Grid Template Browser and select **Properties**. **b** In the Grid Template Properties page, click the Default OneColor protocol row. **c** From the Default OneColor protocol list select **GE1_107_Sep09,** and click **OK.** 	• The software recognizes to use the default 1-color protocol when the 1-color scan file is loaded.

Task 2: Add an extraction set for a 1-color Gene Expression image file of 4-multiplex microarray (continued)

Steps	Detailed Instructions	Comments
3 Add the 1-color GE example image file installed with the Feature Extraction software to Project 1. Note: This image has been renamed from the original scan name. The "14850" in the name corresponds to the AMADID.	**a** Right-click Project Explorer, and select **Add Extraction**. **b** Select the **Hu4x44K_GE1_251485034336_H.tif** file, and click **Open**. **c** Expand the extraction set that appears in Project Explorer. 	• Feature Extraction lets you extract the multiplex images as one image file and produces one .shp shape file for the four microarrays in the multiplex. • This is an XDR image scanned at high and low PMTs. Adding a high PMT scan (_H) automatically loads the corresponding low PMT scan (_L) that is in the same directory. • Feature Extraction automatically loads the grid template which has the default 1-color GE protocol assigned to it. As a result, the protocol is also loaded to the extraction set.

Task 3: Add an extraction set for a CGH microarray image file

Steps	Detailed Instructions	Comments
1 Add the 20-bit CGH image file installed with the Feature Extraction software to Project 1. Note: The grid template for this image is not provided as part of the Example Images installation.	**a** Click the **Add Extraction Set(s)** button on the toolbar, or right-click Project Explorer, and select **Add Extraction**. **b** Select the file **Hu244K_CGH_251469312458.tif**, and click **Open**. **c** Expand the extraction set that appears in Project Explorer. 	• Task 3 shows you how to extract a tiff image without a grid template and protocol attached in the project when you set up the eArray login.
2 Check the Extraction Set Configuration tab to make sure that the assignments are OK for the GE 1-color and 2-color extraction sets.	**a** Expand the columns in the Extraction Set Configuration tab to see all the entries for the three extraction sets.	• Note: The output file name is the name of the extraction set plus the protocol name. • Note: The CGH extraction set does not have a grid template and protocol assigned to it.

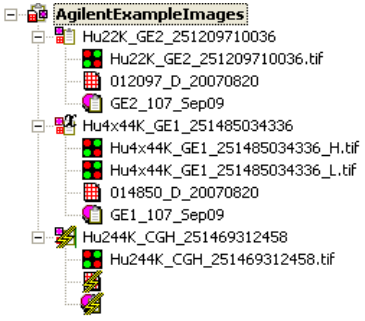
Project Properties | Extraction Set Configuration

Extraction Set Name	Grid Name	Protocol Name	Output Name	Scan File Name	DR 2nd Scan File Nan	Scan File Path
Hu22K_GE2_251209710036	012097_D_20070820	GE2_107_Sep09	Hu22K_GE2_2512097100	Hu22K_GE2_251209710036	<None>	C:\Program Files\Agilent\MicroArra
Hu244K_CGH_251469312458			Hu244K_CGH_25146931	Hu244K_CGH_2514693124	<None>	C:\Program Files\Agilent\MicroArra
Hu4x44K_GE1_251485034336	014850_D_20070820	GE1_107_Sep09	Hu4x44K_GE1_25148503	Hu4x44K_GE1_2514850343	Hu4x44K_GE1_2514	C:\Program Files\Agilent\MicroArra

Task 3: Add an extraction set for a CGH microarray image file (continued)

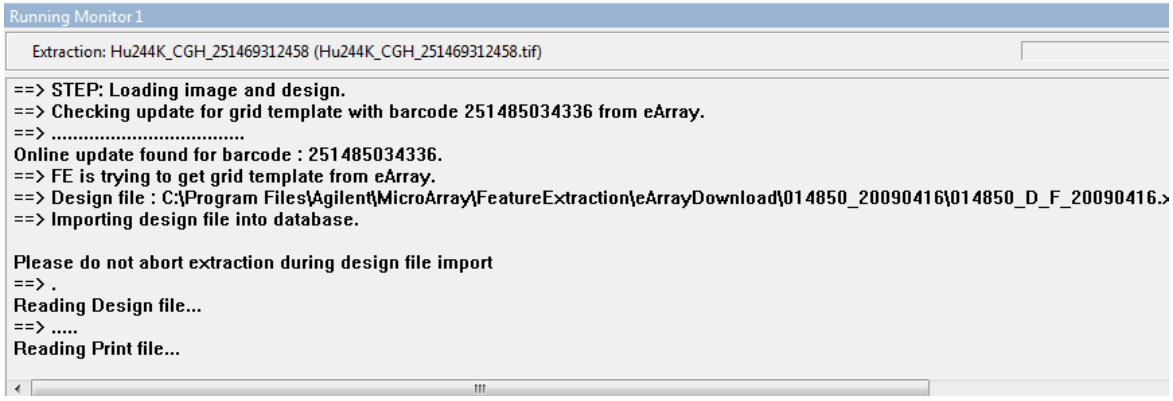
Steps	Detailed Instructions	Comments
3 Enter eArray login settings, if you have not yet done so. You must select the **Use eArray server during extraction** in order for this exercise to work. Note: You must have an eArray account before you can set up the login settings in Feature Extraction.	**a** Select **Tools > eArray Settings**. **b** In the eArray Login Setting window, if you do not have an eArray account, click the Register button to sign up for one. **c** Enter your eArray login information, and click the **Test Connection** button. You should get a message indicating successful login. **d** In **Advanced Options**, select **Use eArray server during extraction**. Click **Save** and then close the eArray Settings dialog box.	• The settings let Feature Extraction automatically log into the eArray server to check for updates of grid templates, protocols, and QC metric sets.

Task 4: Save the project and start the Feature Extraction batch run

Steps	Detailed Instructions	Comments
1 Save the project with a new name.	**a** Select **File > Save As**. **b** Go to or create the folder for your projects, if you want to save in a location different from the default FEProjects directory. **c** Enter **AgilentExampleImages** (or anything you want) in the File Name box, and click **Save**. 	• You must save the project before the program will start extracting. • After you save the project, the project name changes in both the title bar and Project Explorer.

Task 4: Save the project and start the Feature Extraction batch run (continued)

Steps	Detailed Instructions	Comments
2 Run Feature Extraction on AgilentExampleImages project. Note: The Running Monitor window shows the progress of each extraction set.	**a** Click the **Project Run mode On/Off** button, or select **Project > Start Extracting**. The project extraction starts to run.	• In Feature Extraction 10.9 only one extraction set is extracted at a time.


Running Monitor 1
Extraction: Hu244K_CGH_251469312458 (Hu244K_CGH_251469312458.tif)
==> STEP: Loading image and design.
==> Checking update for grid template with barcode 251485034336 from eArray.
==> .....
Online update found for barcode : 251485034336.
==> FE is trying to get grid template from eArray.
==> Design file : C:\Program Files\Agilent\MicroArray\FeatureExtraction\eArrayDownload\014850_20090416\014850_D_F_20090416.>
==> Importing design file into database.
Please do not abort extraction during design file import
==> .
Reading Design file...
==> .....
Reading Print file...

Task 5: View project run summary and QC report

Steps	Detailed Instructions	Comments
1 View the Summary Report tab for the project run summary.	• The **Summary Report** tab appears next to the Extraction Set Configuration tab when the project run starts. • See Figure 6 on page 34 for details.	• Once extraction is done, the Project Run Summary shows information, warnings, errors, and QC reports associated to each extraction set. • For multiplex microarray images, a QC report is generated for each microarray. • Note: The 4-multiplex 1-color GE microarray image will have four QC reports, one for each microarray.

Task 5: View project run summary and QC report (continued)

Steps	Detailed Instructions	Comments
2 Open the QC report for the 2-color GE extraction set, and see whether the results seem reasonable or not. See Chapter 2 in the *Feature Extraction 10.9 Reference Guide* for information on how to interpret the QC Report.	**a** In the Project Run Summary on-screen report, double-click the link to the QC report for Hu22K_GE2. Or do the following: • Go to the folder where the .tif image file is saved. • Double-click the .pdf file for the QC report. **b** Display the grid, spatial distribution plot and plot of median % CV to see any anomalies.	• The QC report (.pdf file) by default is put in the same folder as the image file. You can change the default folder setting by selecting **Tools>Preferences** from the toolbar, and then opening the Output & Data Transfer folder. The default results folder is defined in the Local File Folder setting. • If you are running Feature Extraction with a 30-day demo license, the QC report is an .html file. See Chapter 2 of the *Feature Extraction User Guide* to learn to how print this .html file.

Task 5: View project run summary and QC report (continued)

Steps	Detailed Instructions	Comments

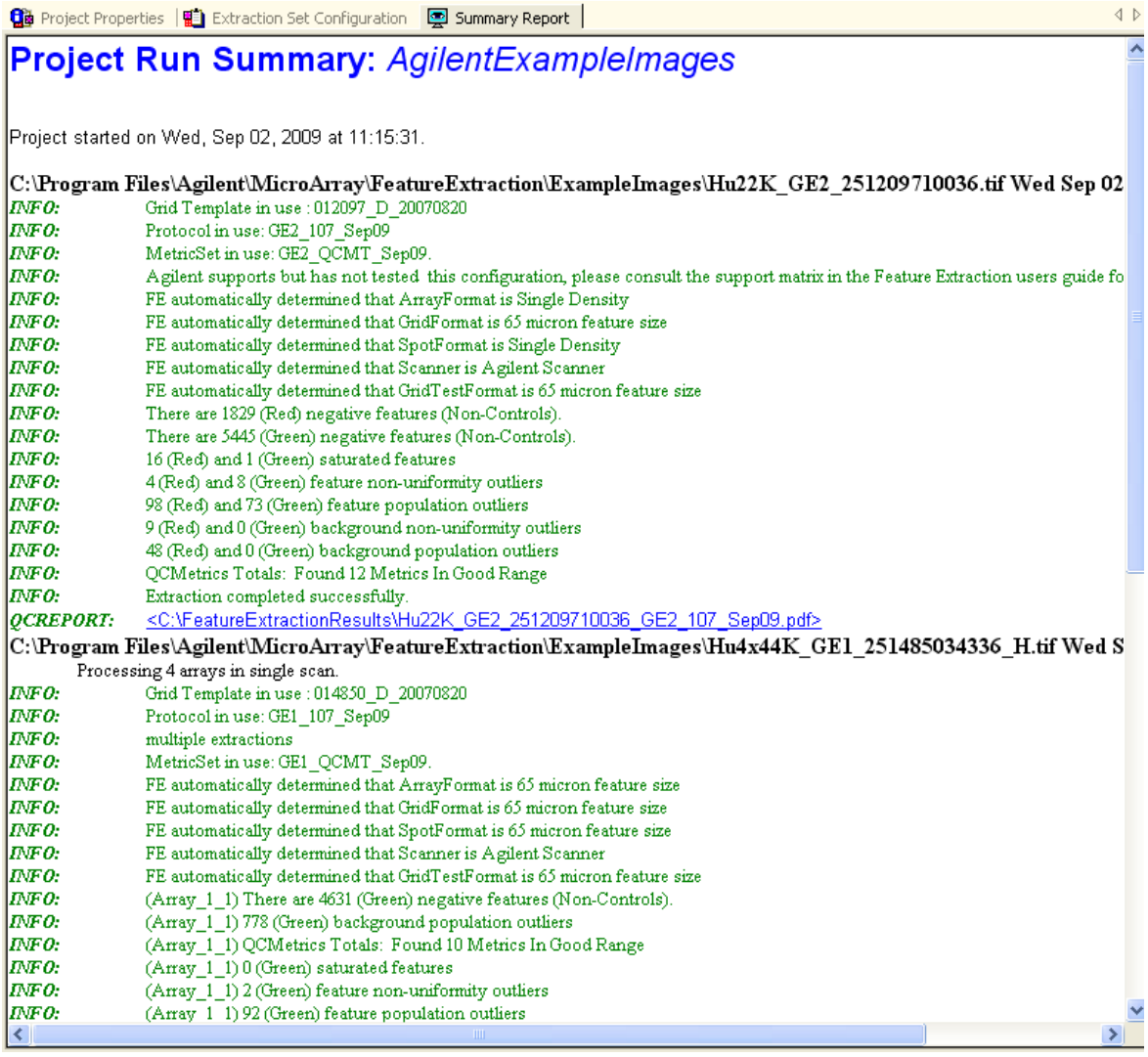

Project Properties
Extraction Set Configuration
Summary Report
Project Run Summary: AgilentExampleImages
Project started on Wed, Sep 02, 2009 at 11:15:31.
C:\Program Files\Agilent\MicroArray\FeatureExtraction\ExampleImages\Hu22K_GE2_251209710036.tif Wed Sep 02
INFO: Grid Template in use : 012097_D_20070820
INFO: Protocol in use: GE2_107_Sep09
INFO: MetricSet in use: GE2_QCMT_Sep09.
INFO: Agilent supports but has not tested this configuration, please consult the support matrix in the Feature Extraction users guide fo
INFO: FE automatically determined that ArrayFormat is Single Density
INFO: FE automatically determined that GridFormat is 65 micron feature size
INFO: FE automatically determined that SpotFormat is Single Density
INFO: FE automatically determined that Scanner is Agilent Scanner
INFO: FE automatically determined that GridTestFormat is 65 micron feature size
INFO: There are 1829 (Red) negative features (Non-Controls).
INFO: There are 5445 (Green) negative features (Non-Controls).
INFO: 16 (Red) and 1 (Green) saturated features
INFO: 4 (Red) and 8 (Green) feature non-uniformity outliers
INFO: 98 (Red) and 73 (Green) feature population outliers
INFO: 9 (Red) and 0 (Green) background non-uniformity outliers
INFO: 48 (Red) and 0 (Green) background population outliers
INFO: QCMetrics Totals: Found 12 Metrics In Good Range
INFO: Extraction completed successfully.
QCREPORT: <C:\FeatureExtractionResults\Hu22K_GE2_251209710036_GE2_107_Sep09.pdf>
C:\Program Files\Agilent\MicroArray\FeatureExtraction\ExampleImages\Hu4x44K_GE1_251485034336_H.tif Wed S
Processing 4 arrays in single scan.
INFO: Grid Template in use : 014850_D_20070820
INFO: Protocol in use: GE1_107_Sep09
INFO: multiple extractions
INFO: MetricSet in use: GE1_QCMT_Sep09.
INFO: FE automatically determined that ArrayFormat is 65 micron feature size
INFO: FE automatically determined that GridFormat is 65 micron feature size
INFO: FE automatically determined that SpotFormat is 65 micron feature size
INFO: FE automatically determined that Scanner is Agilent Scanner
INFO: FE automatically determined that GridTestFormat is 65 micron feature size
INFO: (Array_1_1) There are 4631 (Green) negative features (Non-Controls).
INFO: (Array_1_1) 778 (Green) background population outliers
INFO: (Array_1_1) QCMetrics Totals: Found 10 Metrics In Good Range
INFO: (Array_1_1) 0 (Green) saturated features
INFO: (Array_1_1) 2 (Green) feature non-uniformity outliers
INFO: (Array_1_1) 92 (Green) feature population outliers

Figure 6 Summary report for AgilentExampleImages project

Task 5: View project run summary and QC report (continued)

Steps	Detailed Instructions	Comments

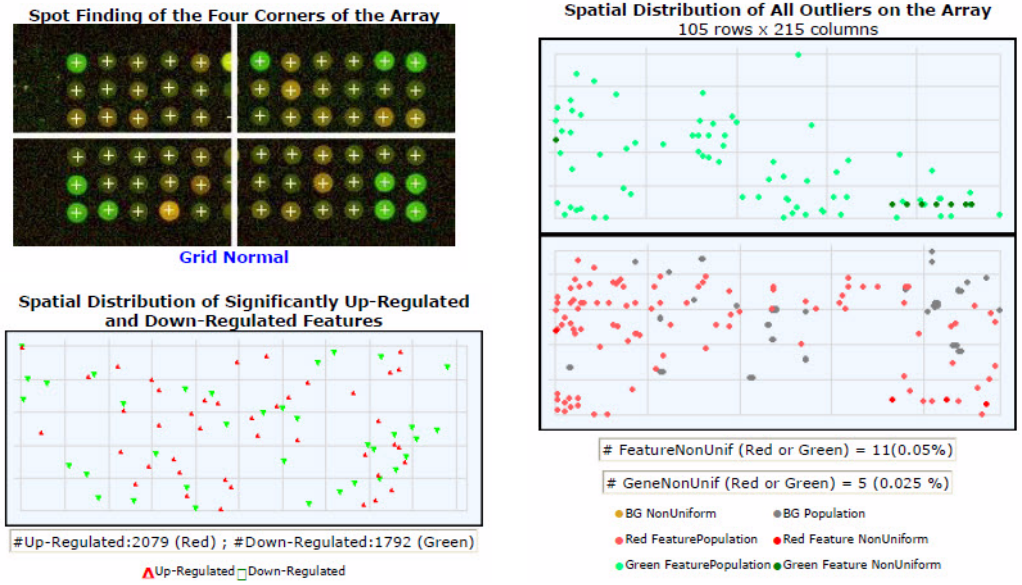
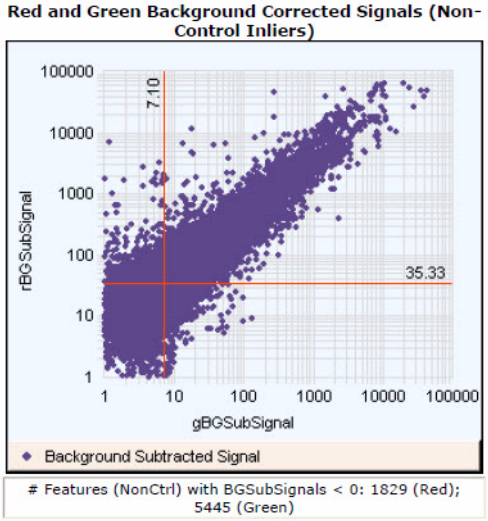

Spot Finding of the Four Corners of the Array
Grid Normal
Spatial Distribution of Significantly Up-Regulated and Down-Regulated Features
#Up-Regulated:2079 (Red) ; #Down-Regulated:1792 (Green)
Up-Regulated
Down-Regulated
Spatial Distribution of All Outliers on the Array
105 rows x 215 columns
FeatureNonUnif (Red or Green) = 11(0.05%)
GeneNonUnif (Red or Green) = 5 (0.025 %)
BG NonUniform
BG Population
Red FeaturePopulation
Red Feature NonUniform
Green FeaturePopulation
Green Feature NonUniform

Figure 7 Grid placement at four corners and spatial distribution plots for Hu22K_GE2 QC report

Task 5: View project run summary and QC report (continued)

Steps	Detailed Instructions	Comments



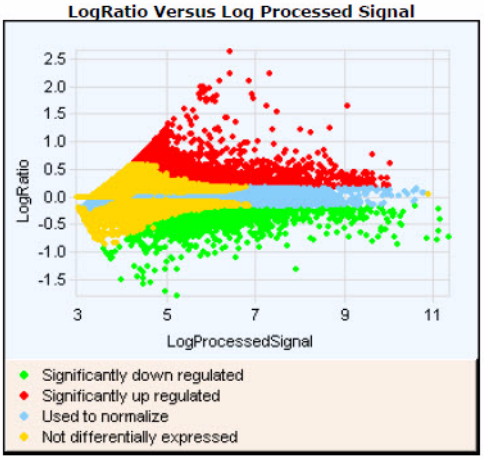
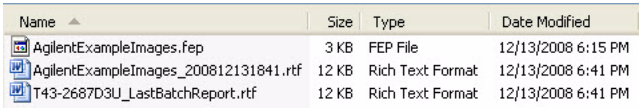


Figure 8 Background corrected signals and log ratio vs. processed signals plots for Hu22K_GE2 QC report

3 Print the latest Project Run Summary report for the project.

a Go to the results folder where the **AgilentExampleImages** project is saved.

b Open and print the _LastBatchReport.rtf with Microsoft Word.



Name	Size	Type	Date Modified
AgilentExampleImages.fep	3 KB	FEP File	12/13/2008 6:15 PM
AgilentExampleImages_200812131841.rtf	12 KB	Rich Text Format	12/13/2008 6:41 PM
T43-2687D3U_LastBatchReport.rtf	12 KB	Rich Text Format	12/13/2008 6:41 PM

- All Summary Reports are saved to the folder containing the project associated with the report.
- Note that there are two reports with the .rtf extension. One is always a specifically-named rtf (ProjectName_Date). The Computer Name_LastBatch file is used for the last extraction run and is overwritten by the next project, if same folder is used.
- If an image was extracted previously, the previous .rtf files are named as Project Name_Date and Time.rtf.

CAUTION Agilent-supplied QC metric sets are intended to assist users in monitoring microarray processing issues. They were not developed to detect microarray manufacturing issues.

Example Exercise 2: Set up and run batch extractions on non-Agilent images

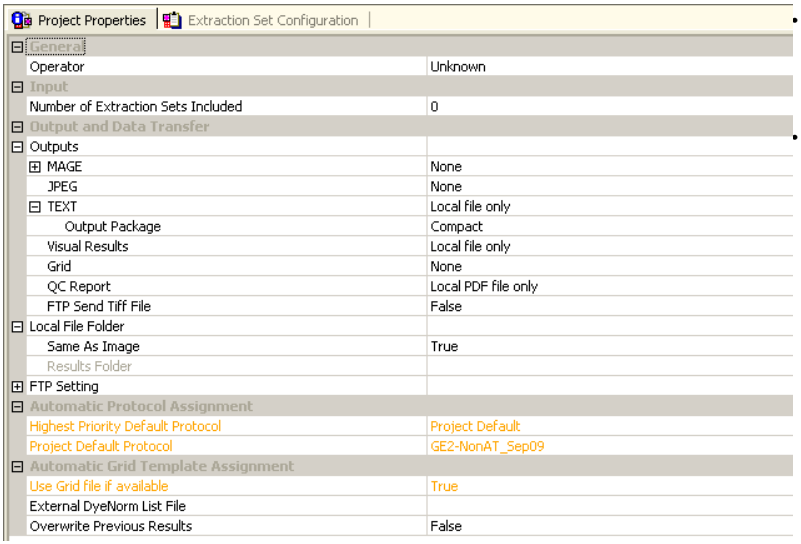
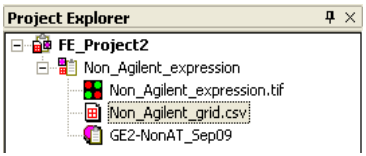
This exercise shows you how to create a new project, change project properties and run Feature Extraction for the non-Agilent image shipped with the software.

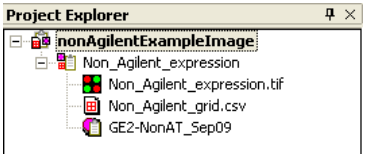
Repeat these instructions to add non-Agilent images of your own to create a batch. You can also set up and run a combination of Agilent and non-Agilent images in one project.

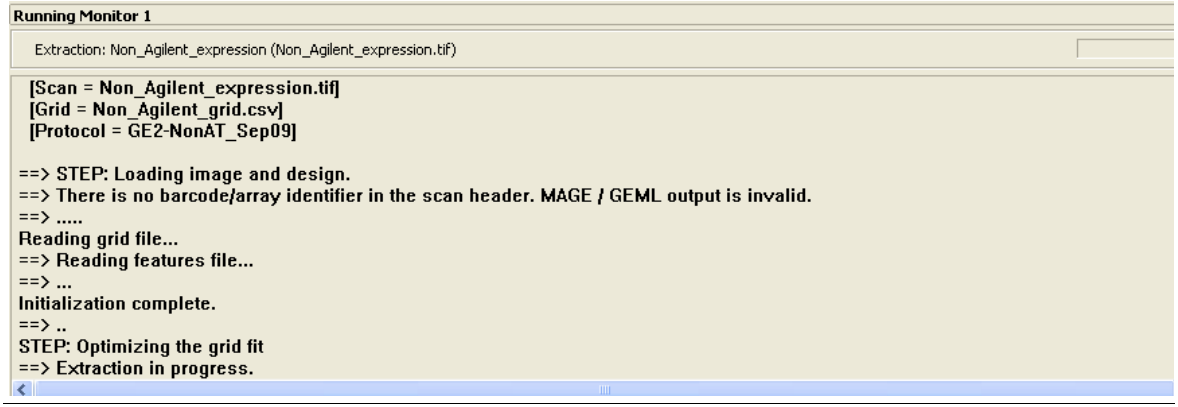
Scans must be from Agilent scanner. Other scanners are not supported.

Try the Steps on the left before reading the Detailed Instructions.

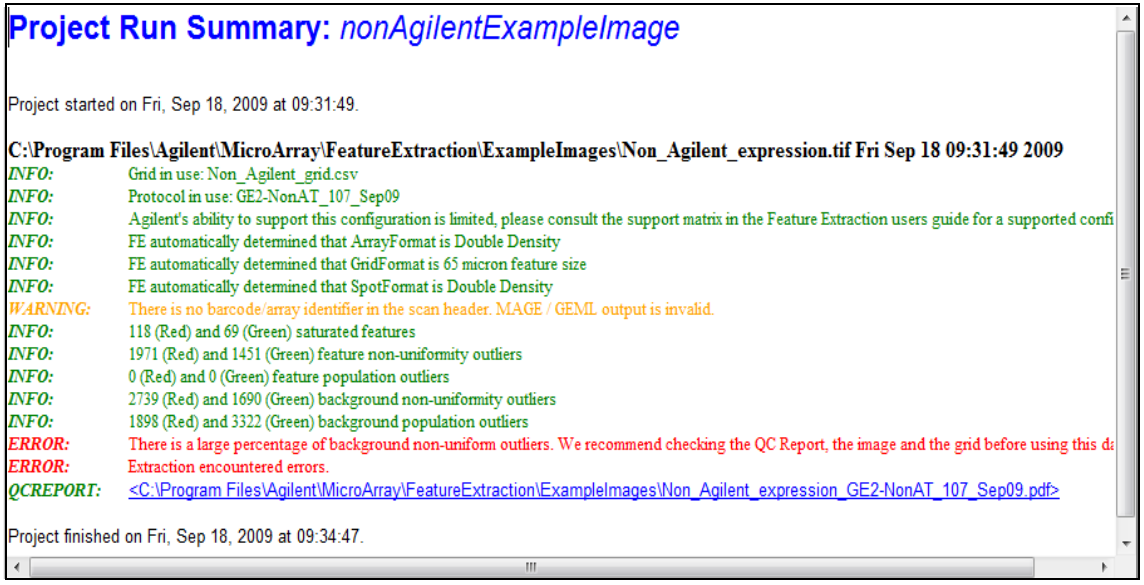
Steps	Detailed Instructions	Comments
1 Create a new standard project. Note: After FE_Project1, the next project you open is FE_Project2. The name of every project that you open after that follows in numerical order, whether you save FE Project N or not or close FE Project N or not.	• Select **File > New > Standard Project**, OR • Click the **New Project** icon, 	• A standard project is one where extraction sets are set up from existing scan files to run automatically as a batch. • Each time you restart the software, the first new project is FE_Project1.
2 Change the following Project Properties: • Highest Priority Default Protocol: Project Default • Project Default Protocol: GE2-nonAT_105_Sep09 • Use Grid file if available: True	**a** Click the **Project Properties** tab. **b** Click the cell next to **Highest Priority Default Protocol**, and select **Project Default**. **c** Click the cell next to **Project Default Protocol**, and select **GE2-nonAT_Sep09**. **d** Click the cell next to **Use Grid file if available**, and select **True**.	• In the Project Properties tab screen, you can select a number of variables that pertain to the project as a whole, rather than to individual extraction sets. • Because some of these properties, such as Project Default Protocol, affect how the extraction set is constructed, you may want to set these before you add the image files (extraction sets).

Steps	Detailed Instructions	Comments
		• See Chapter 2 in the *Feature Extraction 10.9 User Guide* for instructions on how to change other project properties • Note that any changes from the default settings are shown in orange.
3 Add the non-Agilent image shipped with the software to Project 2. • Add any other of your non-Agilent images that you want in the batch.	**a** Click the **Add Extraction Set(s)** button, or right-click anywhere in Project Explorer, and select **Add Extraction.** **b** Go to the directory containing the non-Agilent image, and click **Open**. **c** Expand this extraction set. **d** Right-click grid icon (below the tif file), and click **Select Grid file**. **e** Browse to the folder containing the grid file. For this example, it is located in the same folder as the image. **f** Select the grid file (**_grid.csv**) and click **Open**. 	• The grid is automatically assigned, if its name is the same as the image name and you set the Project Properties parameter "Use Grid File if available" to "True. • Note: In this example, because the the image file name "Non_Agilent_expression" is NOT the same as the grid file name "Non-Agilent", the grid will not be automatically assigned to this image. You must manually select a grid file to use. • The protocol was automatically assigned to the extraction set because you set the highest priority default protocol to be the one for the project and then selected the non-Agilent protocol as the default project protocol.

Steps	Detailed Instructions	Comments
4 Save the project with a new name.	**a** Select **File > Save As**. **b** Go to or create the directory for your projects. **a** Enter **nonAgilentExampleImage**, and click **Save**. 	• For non-Agilent images you must create a grid file (grid.csv) for use in the extraction set. For more information see Chapter 3 in the *Feature Extraction User Guide*.
5 Run Feature Extraction on nonAgilentExampleImage project. Note: The **Running Monitor** window shows the progress of each extraction set.	**a** Click the **Project Run mode On/Off** button, or select **Project > Start Extracting**. The project extraction starts to run.	• In Feature Extraction 10.9 only one extraction set is extracted at a time.


Running Monitor 1
Extraction: Non_Agilent_expression (Non_Agilent_expression.tif)
[Scan = Non_Agilent_expression.tif]
[Grid = Non_Agilent_grid.csv]
[Protocol = GE2-NonAT_Sep09]
==> STEP: Loading image and design.
==> There is no barcode/array identifier in the scan header. MAGE / GEML output is invalid.
==> .....
Reading grid file...
==> Reading features file...
==> ...
Initialization complete.
==> ..
STEP: Optimizing the grid fit
==> Extraction in progress.

Steps	Detailed Instructions	Comments
6 View the Summary Report tab for the project run summary.	• **Summary Report** tab appears next to the Extraction Set Configuration tab when the project run starts.	The Project Run Summary report shows a red Error message that there were a large percentage of background non-uniform outliers. User should check the QC Report and may need to make a grid file to re-extract this image. See the *Feature Extraction User Guide* for information on how to create a grid file.



Project Run Summary: *nonAgilentExampleImage*

Project started on Fri, Sep 18, 2009 at 09:31:49.

C:\Program Files\Agilent\MicroArray\FeatureExtraction\ExampleImages\Non_Agilent_expression.tif Fri Sep 18 09:31:49 2009

INFO:	Grid in use: Non_Agilent_grid.csv
INFO:	Protocol in use: GE2-NonAT_107_Sep09
INFO:	Agilent's ability to support this configuration is limited, please consult the support matrix in the Feature Extraction users guide for a supported confi
INFO:	FE automatically determined that ArrayFormat is Double Density
INFO:	FE automatically determined that GridFormat is 65 micron feature size
INFO:	FE automatically determined that SpotFormat is Double Density
WARNING:	There is no barcode/array identifier in the scan header. MAGE / GEML output is invalid.
INFO:	118 (Red) and 69 (Green) saturated features
INFO:	1971 (Red) and 1451 (Green) feature non-uniformity outliers
INFO:	0 (Red) and 0 (Green) feature population outliers
INFO:	2739 (Red) and 1690 (Green) background non-uniformity outliers
INFO:	1898 (Red) and 3322 (Green) background population outliers
ERROR:	There is a large percentage of background non-uniform outliers. We recommend checking the QC Report, the image and the grid before using this da
ERROR:	Extraction encountered errors.
QCREPORT:	<C:\Program Files\Agilent\MicroArray\FeatureExtraction\ExampleImages\Non_Agilent_expression_GE2-NonAT_107_Sep09.pdf>

Project finished on Fri, Sep 18, 2009 at 09:34:47.

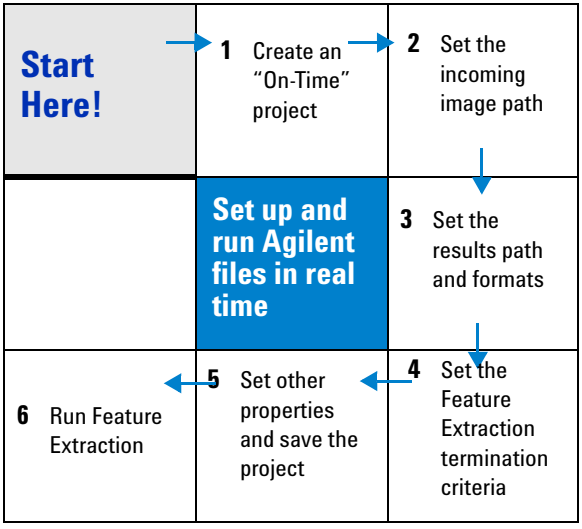
7 View the QC Report	**b** Double-click the blue line containing the link to the QC report.	On the QC report, note the red **Evaluate Grid** message under Spot Finding of the Four Corners of the Array.

Setting up an On-Time project to extract Agilent image files in real time

Instructional overview

An On-Time project lets you set up the Feature Extraction software to automatically extract Agilent microarray image files as they are transferred from the Agilent scanner to a folder of your choosing.

Follow the workflow below and the instructions on the next pages to get started.


Start Here!
1 Create an "On-Time" project
2 Set the incoming image path
Set up and run Agilent files in real time
3 Set the results path and formats
4 Set the Feature Extraction termination criteria
5 Set other properties and save the project
6 Run Feature Extraction

NOTE On-Time projects work only with Agilent microarrays scanned on the Agilent scanner.

General Instructions

These instructions help you set up the program to run a batch of Agilent microarray images in real time as they arrive from the Agilent scanner to a folder of your choosing. These instructions do not include running Feature Extraction.

Step 1. Create an on-time project

- Select **File > New > On-Time Project**.

  Note that you do not add extraction sets to on-time projects.

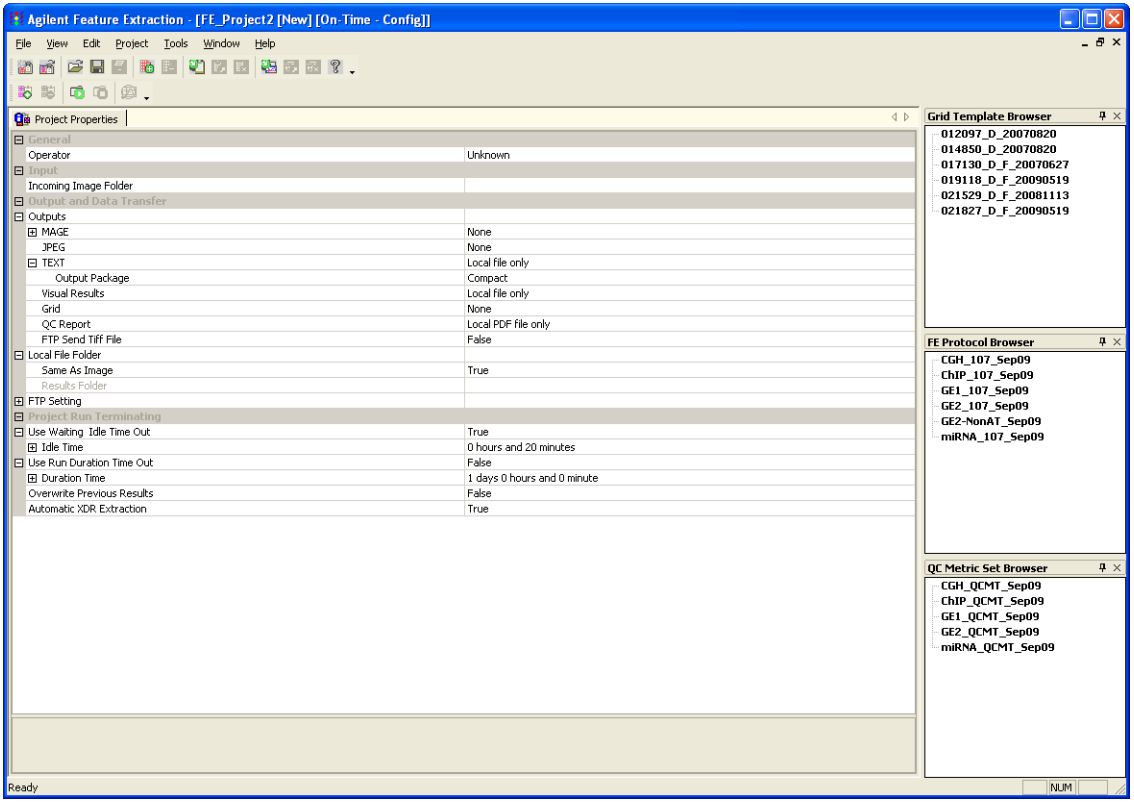


Figure 9 On-Time Project Properties window

Step 2. Enter the incoming image path

1 Click the cell next to Incoming Image Folder, and select the path for your scanned images.

2 Click **OK**.

The software assigns the appropriate grid template to each of the Agilent microarrays and the appropriate protocol included in the Grid Template Properties sheet.

If you want the On-Time project to process microarrays whose grid templates are missing from the database, you must set up the software to use the eArray server during extraction. The required grid templates, protocols, and QC metric sets are automatically downloaded and installed in the database. See "To set up eArray login" on page 11 for more details.

Step 3. Enter termination criteria

1 Click the cell next to Use Run Duration Time Out, and select **True**.

2 If not expanded, expand the Duration Time folder.

3 Use the sliders to enter 0 for **Days**, 16 for **Hours** and 47 for **Minutes**.

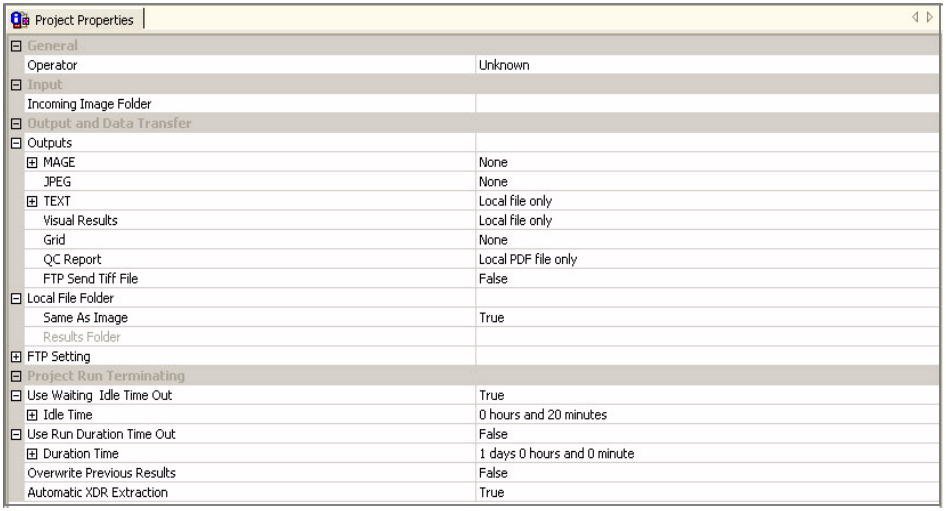


Figure 10 On-Time Project run terminating

Step 4. Change QC metric set associated with a protocol to monitor QC metrics

To change the QC metric set associated to the Feature Extraction protocol:

1. In the FE Protocol Browser, double-click a protocol to open the FE Protocol Editor.
2. Select the **Protocol Properties** tab to see the protocol properties.
3. Under General, click the cell next to **Metricset**.
4. Click the down arrow, and select the QC metric set appropriate for your microarray application.

   If a metric set you want to use is not available in the metric set list, you must first import it to the database.

   To import a QC metric set into the database:

   1. Select **View > QC Metric Set Browser** from Feature Extraction main window, if it is not already visible.
   2. Right-click in the QC Metric Set Browser and select **Import** to import either Agilent metric sets or metric sets created with the QC Chart Tool.

   The default QC metric sets installed with the Feature Extraction software are read-only and can not be removed from the database. But you can remove a QC metric set from the protocol.

   To remove a QC metric set associated with the protocol:

   1. Select **<Set empty>** from the metric set list in the Protocol Properties.
5. Click **Save As** to save the new protocol.

Step 6. Save the project

1. Select **File > Save As**.

   By default, your projects are saved in **C:\Program Files\Agilent\Agilent Genomic Workbench Standard Edition <version>\FeatureExtraction\FEProjects.**
2. To save in a different location, go to or create the folder for your projects.
3. Type a project name, and click **Save**.

Step 4. Run Feature Extraction

1 Make sure that the scanner is ready.

2 Click the **Project Run mode On/Off** icon in the Feature Extraction Project Tools, or select **Project > Start Extracting**.

The image files are saved to the Incoming Image Folder as they complete scanning and are then extracted automatically.

You can display the progress of the extractions, the Summary Report, and the QC Report as you did for the Standard Project extractions.

The Summary Report notes how many metrics are within range for each extraction. The QC report for each image contains the values for the metrics and an evaluation of whether they were within or exceeded the threshold limits.

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In this book

This book contains brief instructions to help you get started with your Agilent Feature Extraction 10.9 software. This book shows you:

- What is new for version 10.9
- How to set up and run batch extractions of existing Agilent and non-Agilent image files
- How to set up and run batch extractions of Agilent image files in real time

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